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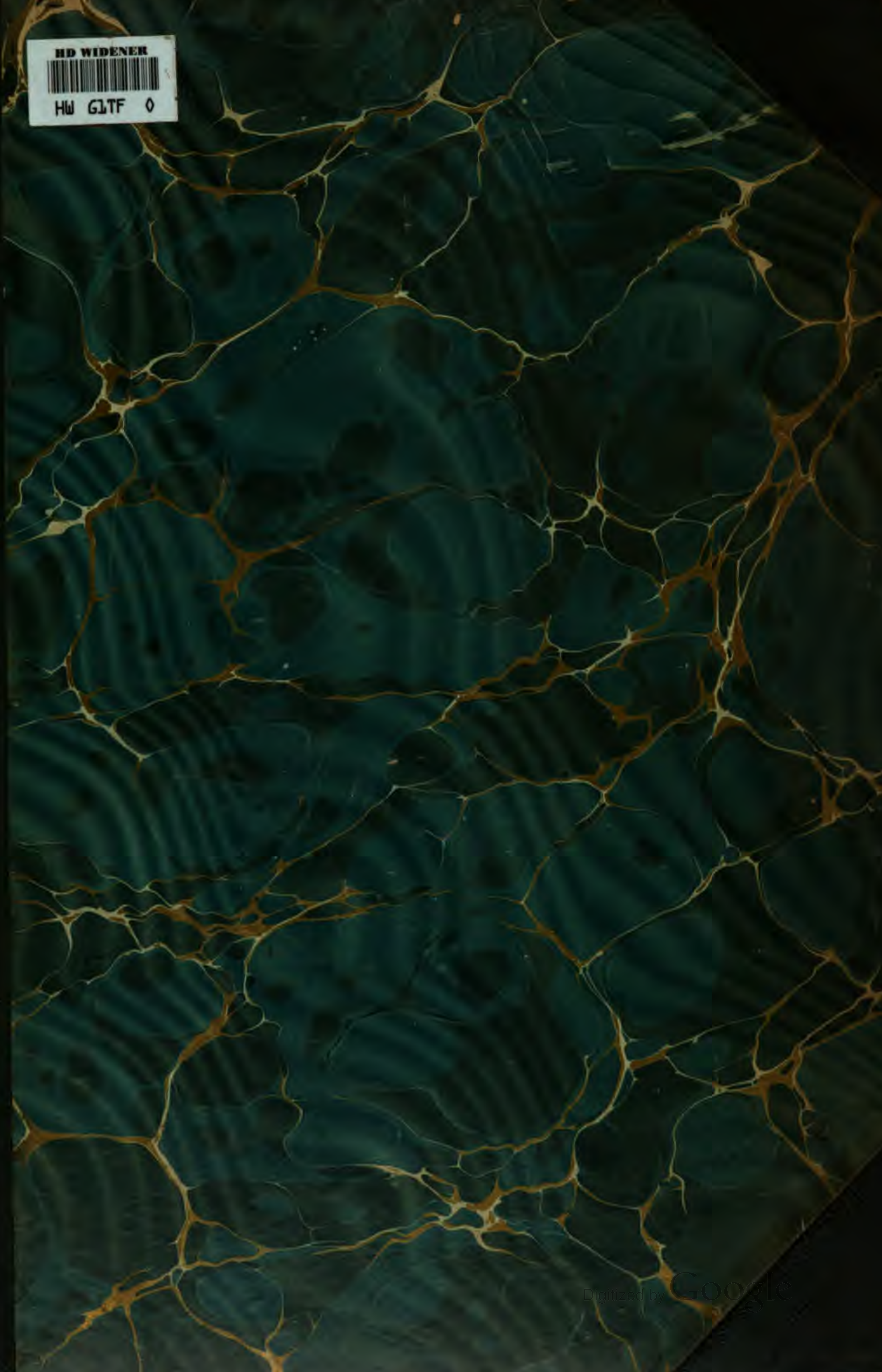

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Commissioners of Fisheries, Game and Forests

OF THE STATE OF NEW YORK.

Report Upon the Recent Epidemic Among Brook Trout *(Salvelinus fontinalis)* on Long Island.

By

Gary N. Calkins.

1898.

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Report Upon the Recent Epidemic Among Brook Trout *(Salvelinus fontinalis)* on Long Island.

By GARY N. CALKINS.

HOLDING HARD.

IN May, 1899, an epidemic started among the brook trout in the hatcheries of a trout farm at Northport, Long Island, and did not abate until every fish had died. The painstaking care taken by the State Shellfish Commissioner, Mr. Edward Thompson, during the last ten years in developing an excellent hatchery and in raising a fine breed of trout, thus in one season received a serious check. At the request of the State Fish Culturist, Mr. A. Nelson Cheney, I undertook an examination of the fish for the purpose of ascertaining the direct cause of the epidemic, and if this were due to a parasite, of finding out something of its life history and affinities. Thanks to the courtesy of Mr. Cheney and Mr. Thompson, I was able to get abundant material.

With the exception of the fungus *Saprolegnia*, which does not menace the life of the fish, the brook trout has apparently been very free from disastrous epidemics. In the literature at my command I find no mention of *Salvelinus fontinalis* or *Salmo fario*, as subject to any particular disease, and, to my knowledge, the only statement of a parasite in these forms, was made by Csokor in 1888, in a short description of Gregarinosis.* About ten years ago an epidemic killed off hundreds of the trout in the New York State hatcheries at Cold Spring Harbor, Long Island, but the cause of the trouble was not located and no report was made. From various sources, however,

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* (Gregarinosis d. Forellen, *Oesterreich. Zeit. f. wiss Veterinark.* Wien 1888, 11, p. 56-58.)

I have obtained enough evidence to lead me to the conclusion that the epidemic at Cold Spring was quite similar to the present one and, if my surmise is correct, it is a significant fact that the same trouble should have occurred in two hatcheries in the same region.

Parasitic diseases are not uncommon among fish and the so-called psorosperms, in particular, have been recognized as disease-causing organisms since 1841, when Johannes Müller first called attention to them. These have since been called the Myxosporidia by Bütschli and other students of the Protozoa, and are now known to be minute unicellular animals which by the accumulation of spores, form great cysts in the muscles and connective tissues of fish. These are the most destructive parasites known to the fish-breeder, and in some cases great epidemics are due to them. During an epidemic among the barbels of the Meuse, in 1883 to 1885, hundreds of fish died every day from this cause. The Myxosporidia are such frequent parasites and so often the cause of fatal diseases in fish that it is not remarkable that I confidently expected to locate the cause of the epidemic in some organism belonging to this group. In this, however, I was disappointed, for the first glance at the diseased fish showed a complete absence of cysts or tumors which characterize many of the more common parasites. Although tumors were absent, the body was frequently ulcerated and great holes in the body walls were often present, while smaller holes were quite characteristic. As this condition frequently accompanies Myxosporidiosis, I was still confident that the organism could be readily determined upon sectioning some of the ulcerated spots. When this was done there were still no traces of Myxosporidia, but instead of them, I found myriads of minute forms belonging to the same group as the Myxosporida, *i. e.*, to the Sporozoa, but which cannot be classified among the usual fish parasites. This parasite, which has never been described, I shall name *Lymphosporidium truttæ*, and in the following report I will give as much of its life history as could be made out.

Characteristic Symptoms of the Disease.

The epidemic was equally fatal to fish of all ages. Mr. Thompson estimates that 2,000 yearlings, 1,000 two and three year old fish, and some 10,000 "fingerlings" (from four to six inches long), were lost. They died as rapidly during the cold days of November as in the hot days of July and August; indeed, the ponds are fed by springs and Mr. Thompson asserts that the temperature of the water never rises above sixty degrees F.

The fish, especially the yearlings, were characterized by sluggish motion and inability to withstand rough treatment of any kind, most of them dying within a few minutes after removal from the waters of the runways to a pail. In the water the

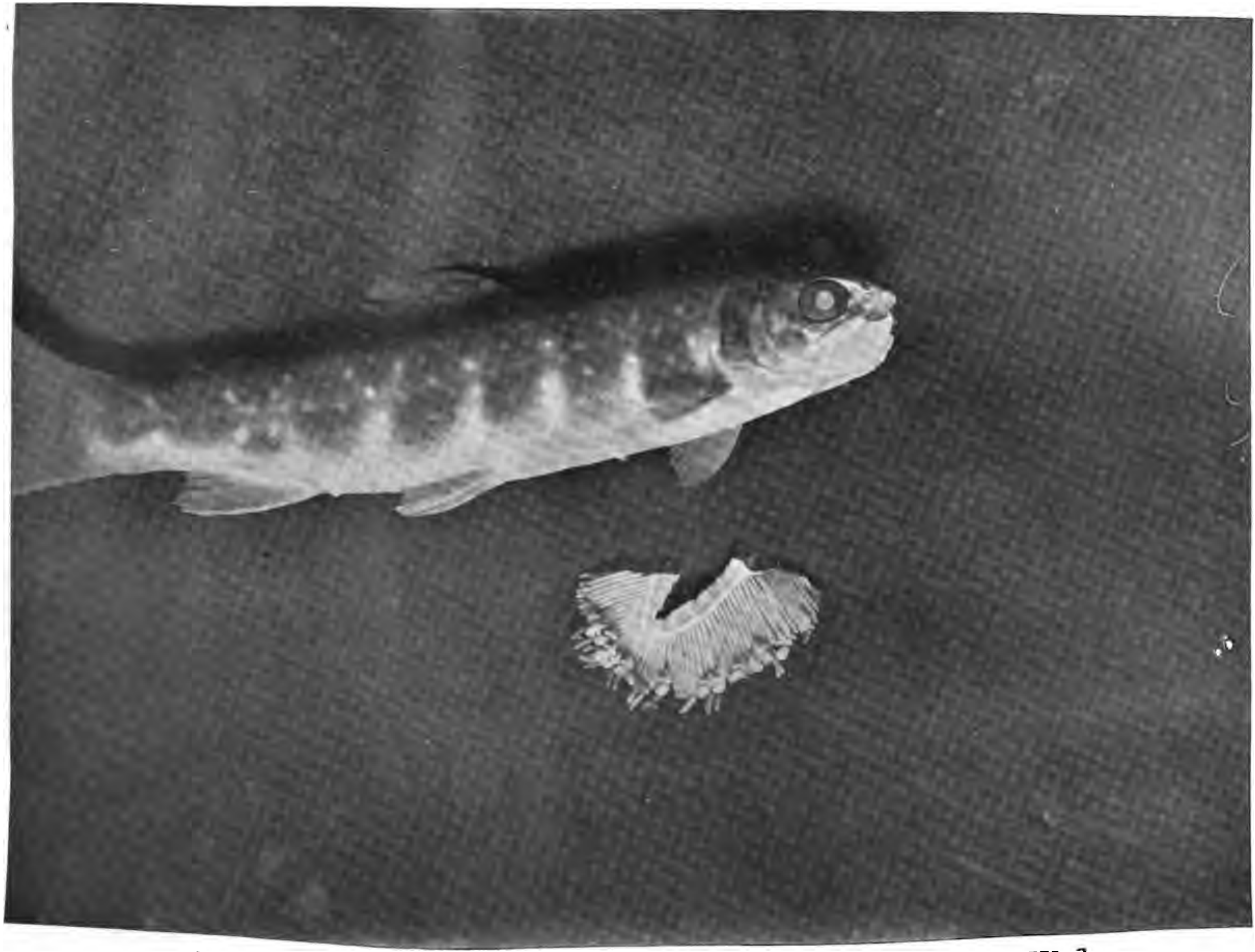

PLATE I.—YOUNG BROOK TROUT, DISEASED.—GILL OF LARGE FISH WITH COPEPOD PARASITES.

actions of the fish were indicative of their diseased condition, for they would frequently rise to the surface and swim upon the side or turn belly-up on the surface, while at one period hundreds of the dead fish were daily removed from the runways. In some of these there were no external indications of the disease; in others, and in the majority of the cases, great red spots or ulcers were visible, sometimes on the belly, again on the back, but most often on the sides. The bases of the fins were particularly subject to these sores and the eyes were often disfigured by them. More serious disfigurements were frequently seen in the entire loss of one half of the lower jaw, bone and tissues being eaten entirely away (Plate I); or, again, the ulcers in the sides would give rise to holes through the body wall and into the body cavity, and live fish were occasionally seen with parts of their viscera hanging out of the holes thus made (Plate II). The red ulcers which are due to the congestion of the blood at the sore spots give place to wide gaping wounds sometimes an inch in diameter where the flesh has dropped out (Plate II). In some cases the vertebræ in the caudal region were thus exposed. In many cases the holes or sores did not go entirely through the body wall but formed shallow and irregular wounds. These sores were most apparent on the small yearlings; the older fish, however, were not exempt but in these cases the sores were proportionately smaller and distributed in all regions of the body.

Methods Employed in the Investigation.

The wide distribution of the sores about the animal, from the posterior end of the body to the eyes and jaws, was sufficient evidence that the cause of the disease was pretty well distributed throughout the organism, and if further evidence was necessary, it was furnished by the cases mentioned above where the fish were found dead with no external sores of any kind. It was at once apparent from these facts that the cause of the trouble was of some deep-lying nature and that, if parasites were at the bottom of it, they must be widely distributed among the various organs in the body and probably carried to all parts by the blood and lymph circulation. The various organs of diseased fish were therefore cut into small pieces and preserved in diverse killing agents, including sublimate acetic, (saturated corrosive sublimate with five per cent. glacial acetic), saturated corrosive sublimate in normal salt solution, and Flemming's fluid (osmic acid, chromic acid, and glacial acetic acid in certain definite proportions) The organs thus preserved included the testis, kidney, ovary, gall bladder, pyloric cœca, digestive tract (including stomach and intestine), liver, gills, and sore spots in the outer wall. These were taken to the laboratory, where they were sectioned in paraffine and stained in various ways, the most satisfactory stain being iron hæmatoxylin with a counterstain of orange or eosin; the Flemming triple stain of safranin, gentian violet and orange also gave very good results as did thionin and eosin.

There was little or no chance to try direct inoculation for, so far as could be seen, all of the fish in the hatchery were affected by the disease at the time my attention was called to it. One experiment, however, was tried. Some fresh trout were obtained from the northern part of the State and three of them were fed with bits of the flesh of diseased fish containing the ulcerated spots. All of these fish died within three months, probably from the same disease that killed off the other trout, but I had no chance to examine them.

The Specific Cause of the Disease.

The cause of the trouble is, I believe, a sporozoan parasite and the life history of the organism is presented in the present report. I hope to be able at a future time to complete the study begun here and to perform conclusive experiments upon inoculation. Owing to the failure to keep the fish alive in aquaria these experiments have not been attempted during the present winter.

The brook trout, like all others of the same family, is subject to the growth of the fungus *Saprolegnia*, but in the Northport hatchery this has never become a menace and has been easily taken care of. Other parasites which were found, especially in the larger fish, represent different groups of the animal kingdom. Among these were Nemathelminthes, or round worms, and parasitic Copepoda or Crustacea. The round worms belong to a group—the Nematoda—which are very common in fish of different kinds, as well as in all other forms of Vertebrata. They were found chiefly in the swimming bladder and were present in considerable numbers and in all stages of development. They belong to the genus *Ascaris* of which there are 102 distinct species, and only a very few are known to produce fatal results. Each female individual produces thousands of eggs which are retained until the embryo has reached a considerable size. Each egg is surrounded by a thick capsule and is capable of resisting heat or chemicals for a considerable time. They are passed out of the body either through the mouth or the anus and ultimately get into a new host possibly after passing through an embryonic period in some lower form such as an insect or a mollusk.

The parasitic Copepod, *Tracheliastes* sp., is also frequently found upon freshwater fishes where, especially in old or in diseased fish, they become attached to the gills, sometimes in great numbers (Plate I, figure of gill). When young, these parasites bore into the soft tissues of the gill, where they retain their position by an

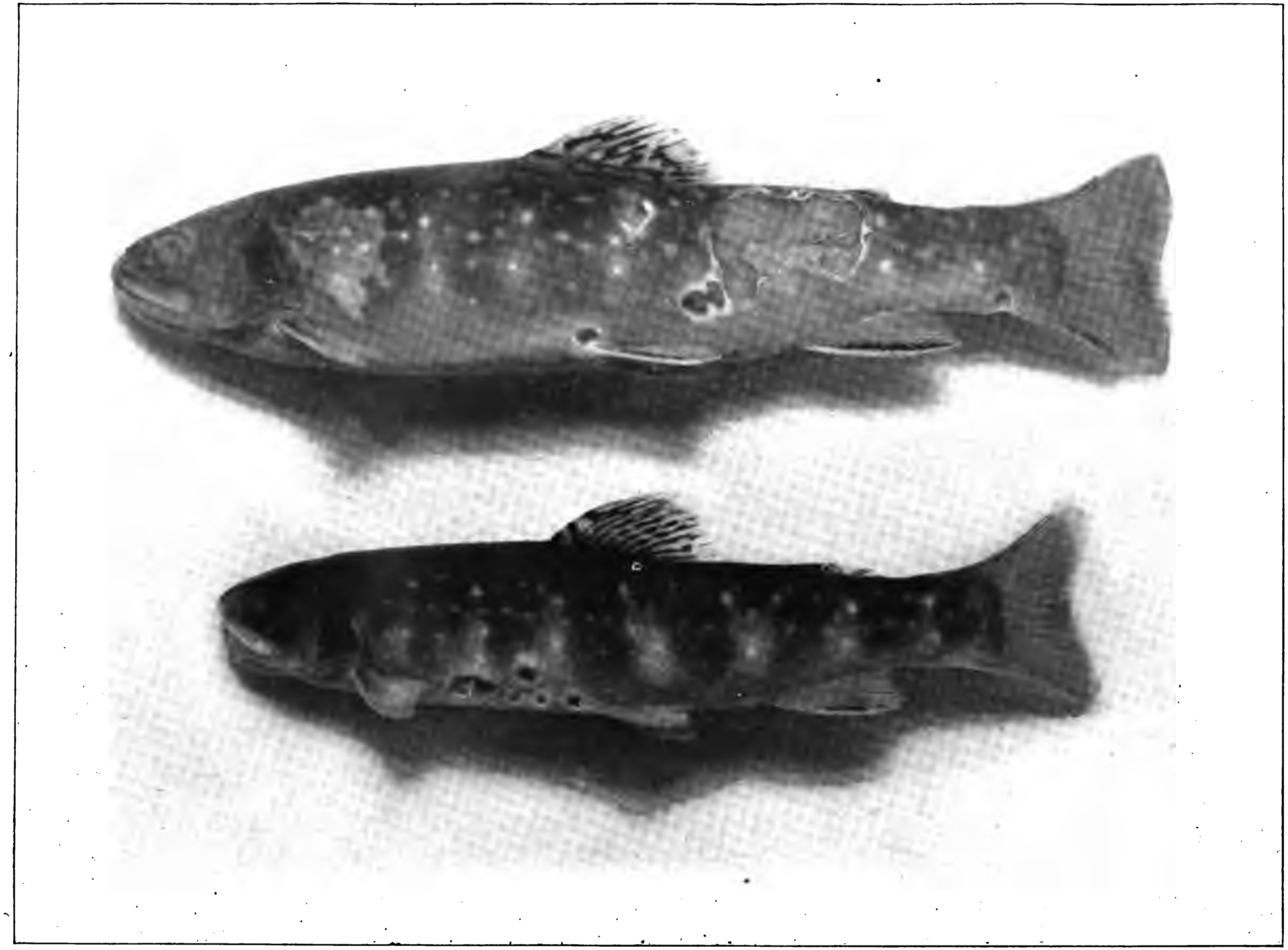

PLATE II.—DISEASED FISH WITH ULCERS.

anchor-like enlargement at the extremity of the anterior appendages. The mouth is adapted for sucking and the parasite is nourished by the blood obtained from the gills.

None of these parasites were numerous enough to account for the trouble, and, although an occasional intra-cellular sporozoan parasite was found, they were not abundant enough to warrant continued search, for in fish with the most noticeable external indications of the disease, the organs within were apparently normal and cysts or other usual indications of Sporozoa were not forthcoming. In every section that was cut, however, a variable number of minute foreign bodies, which were so small that they were at first taken for bacteria, were found especially abundant in the lymph spaces surrounding the various organs and in the testis. These were so minute that it seemed hardly possible that they could be the cause of the disease and yet their numbers were so constant and they were so widely distributed throughout the entire body that I was forced to the belief that they were the cause of the epidemic. They were found in the body cavity surrounding the intestine and other visceral organs, in the lymph spaces, in many of the organs themselves, in the blood vessels including capillaries and veins, and in the gills, muscles, and connective tissue of the diseased fish. In short, they were found wherever there was a cavity, sometimes only occasionally, again in great multitudes.

As there were no satisfactory experiments in inoculating normal fish to show that this organism is the cause of the disease, I will give a brief description of each of the important organs of the body showing the appearance of the sporozoan in question and its wide distribution. The organs chosen are the intestine, including the pyloric cœca, the liver, kidney, gall bladder, blood vessels, testis, and muscles, especially those around the ulcers in the body wall. All of the organs were taken from young fish of not more than five inches in length, and all of the fish showed external evidences of the disease.

The stomach and pyloric cœca had only an occasional spore of the parasite but the intestine (Plate III) had many of them mixed in with the bacteria. Here, too, they were in reproductive stages and it was plainly evident that they had been taken in from the outside through the mouth. There is little doubt that this is the means of infection from fish to fish.

The liver showed no traces of disease either macroscopically or microscopically, the cells being perfectly normal and with no parasites among them. The kidney, on the other hand, frequently appeared swollen and discolored while numerous scattered parasites were seen in it, especially at the anterior end, where the tissue is more lymphatic than in the posterior part and has no excreting function. Only a few parasites were observed in the posterior region of the kidney and these at such rare intervals that the organ could scarcely be said to be diseased. I regard the discoloration of this organ as due to the general morbid condition of the fish rather than to a specific cause.

The spleen, unlike the liver, contained a varying number of parasites although they were never abundant enough to cause distortion of the organ nor to give to it a pathological appearance. They never appeared here in groups as in the intestine and their presence in this organ is probably accidental. The gall bladder contained a great number of spore-forming individuals but only a few of the free spores. Unlike the other organs, the testis contained countless numbers of the parasites and thick masses were formed, in some cases completely filling up the lobes of this organ, which thus appears to be the main seat of the disease. It was greatly enlarged, while the gonadel cells were abnormal and for the most part degenerate (Plate IV).

Among these various organs of the body, therefore, the only one which was found to harbor enough parasites to do injury was the testis, while, with the exception of the intestine, no other organ contained more than a few scattered ones. The outsides of all of the organs, however, that is, the surfaces which are exposed to the body cavity and its fluids, were in all cases covered with the parasites which, especially in the lymph spaces, frequently formed thick masses (Plates V, VI and VII).

The immense numbers of parasites in the testis, the body cavity, and in the lymph spaces, show that these are the principal seats of the organism. They are never absent from the body cavity and they settle upon all of the organs within it including the mesentaries, the fat bodies, and blood vessels as well as all of the organs mentioned above. From here, also, they are carried to all parts of the body and especially to the muscles of the body wall which are bathed in lymph. Here they penetrate the muscle bundles and accumulate in the spaces between them until the cavities are entirely blocked up (Plate VIII). This is, I believe, the cause of the ulcers and ultimate perforations in the body wall, the trouble being brought about by stoppage of the food supply, and the tissues finally disintegrate because of the lack of nutrition. The early stages of the localized trouble are always characterized by congestion of the region about an ulcer, the blood vessels at such places being gorged with blood. Not only the muscles but the cartilages also are apparently affected by the same conditions as seen in the loss of certain bones in the head of some fish (Plate I). The disease can be compared with leprosy in man where the bacteria which cause this disease are known to accumulate in the blood vessels and block up the normal food channels, causing abnormal growths or atrophy of the existing tissues through lack of nourishment, and so leading to ulcers and other external evidences of the disease, and even to the loss of bones.

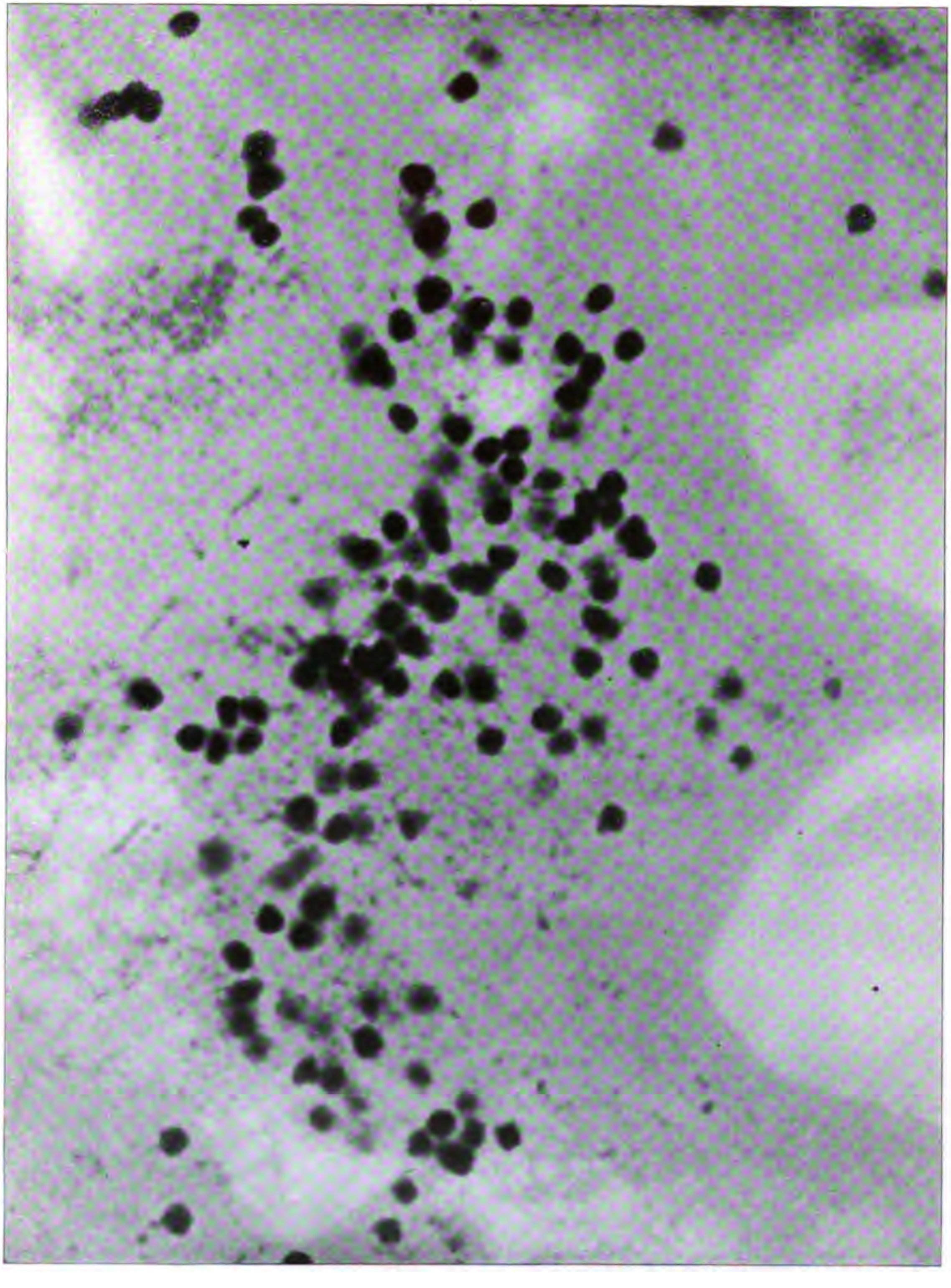

PLATE III.—SPORES OF LYMPHOSPORIDIUM TRUTTÆ IN THE INTESTINE.

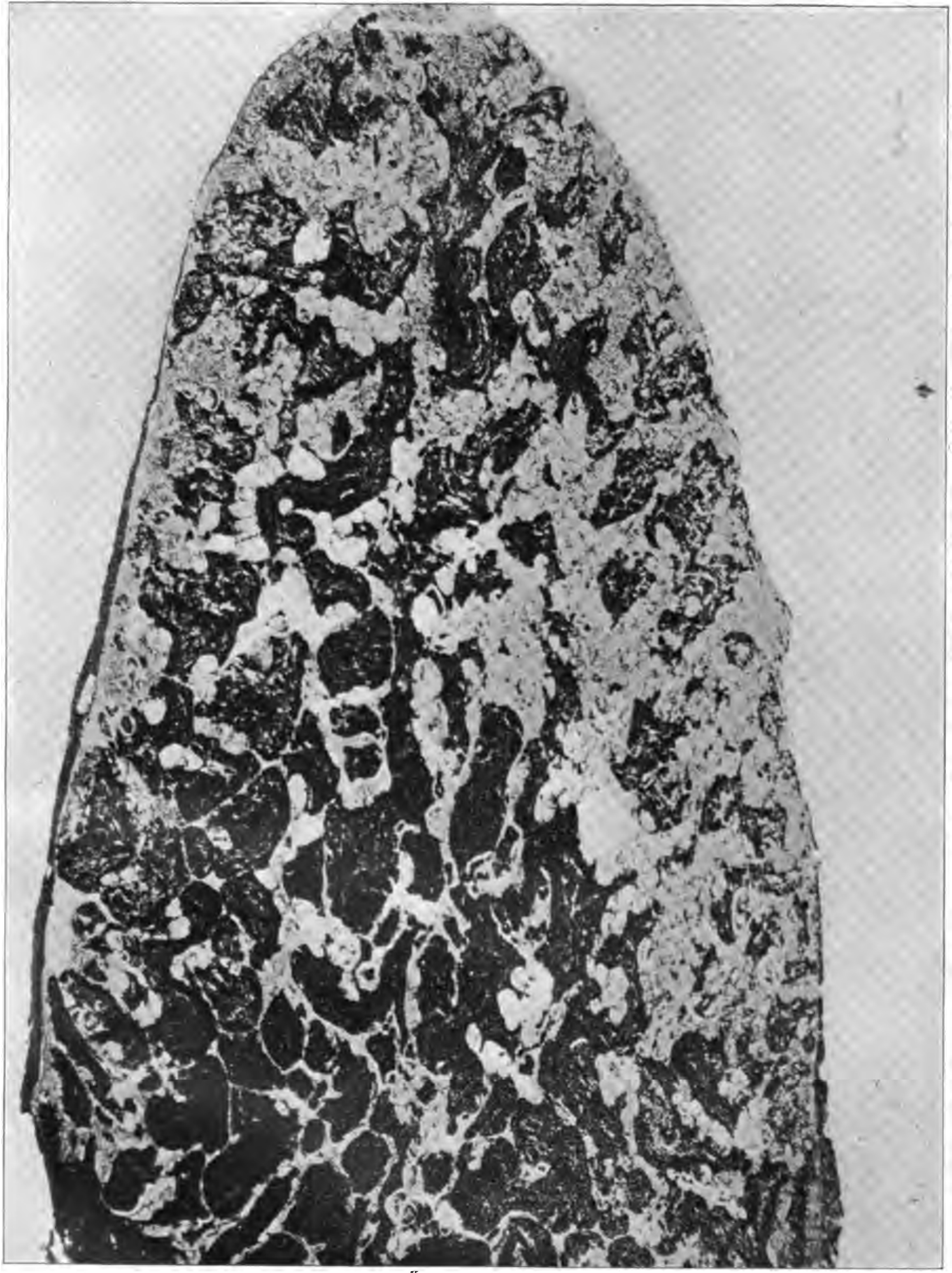

PLATE IV.—SECTION THROUGH THE TESTIS OF A DISEASED FISH.

Description and Life History of the Parasite.

In all Sporozoa where the life history is fully known, the adult individuals give rise to a definite number of spores. Instead of developing at once into new organisms, these spores in turn give rise to progeny by dividing into a definite number of parts. The germs formed by this second division are called sporozoites and each is capable of developing into a new adult, and of repeating the cycle. The parasites in the spore stage are frequently taken into the stomach with the food. Here the digestive fluids dissolve the spore cyst and liberate the sporozoites which penetrate the epithelial cells and grow to full size. After a certain number of such cycles, however, there is a period of conjugation in which two individuals fuse together. In some unknown way the fusion leads to a renewal of vitality and, indirectly, to reproduction by spore-formation for a number of generations.

In the present investigation I have been able to follow the life history of the parasite which, I believe, is the cause of the present epidemic, and to which I have given the name *Lymphosporidium truttæ*, from the sporozoite stage, until sporozoites were again formed. But I have seen no trace of conjugating individuals and this page of its life history, for the present, at least, must remain closed.

I will begin the description of the life history with the spore stage. The spores are pyriform in shape but flattened upon the broader end. Under ordinary conditions of fixation and staining they appear homogeneous and without internal structures of any kind; they always stain intensely with the nuclear stains (basic stains). I was unable to determine whether the homogeneous appearance indicates a similar condition throughout the entire cell or whether it was due to incomplete extraction of the stain. In many cases, however, preparations were obtained in which the organism was differentiated into a peripheral deeply staining portion and a less stained part with a central, nucleus-like body. This condition, however, marks a stage in the life history and indicates the preparation for sporozoite-formation, while the intense homogeneous appearance indicates a young form or an unripe spore. In size the spores are never larger than 2.5 microns (.0025 mm., or .0001 inches) and never smaller than 2 microns. In some conditions, especially during and before sporozoite-formation they lose their pyriform shape and become circular in outline.

Great bunches of spores, as described above, are found in the intestine, and in such groups there are, here and there, certain individuals in which the body is divided up into eight parts. These parts are the sporozoites and in some cases, in certain divisions of the intestine, all of the individuals of a group are in some stage of sporozoite-formation. The first indication of the process is a noticeable cleft beginning at the broad end of the spore, while the entire periphery appears irregular and minutely lobed. The mass of protoplasm segregates into eight small spheres which are not confined by a membrane. The method of formation of these reproductive bodies could not be determined owing to the extremely minute size although various stages were seen, and it was conclusively proved that the groups of eight spheres were derived from single spores. Each sporozoite at this stage measures less than one half of a micron (.0001 inches). (Figure 1, J and K.)

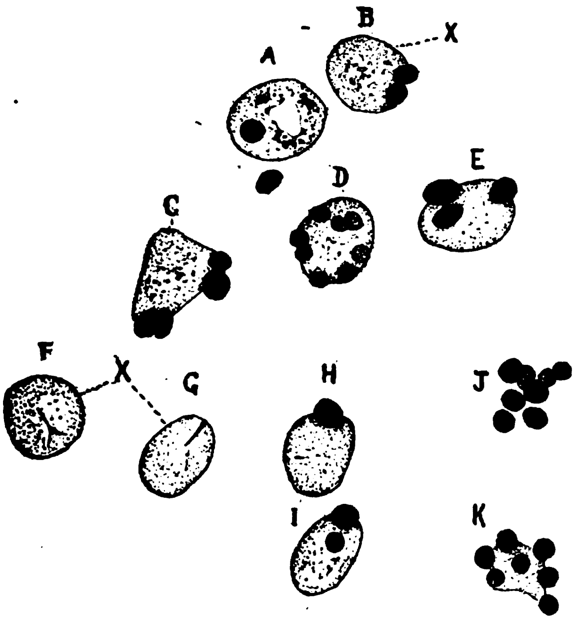

FIGURE 1.—A group of spores from the testis (A to I), and from the intestine (J and K). The cyst (X) is absent in the latter. Camera drawing, x 4000 diameters.

A similar process of sporozoite-formation was seen in the spores infesting the testis where, in one of the fish examined, almost every spore was in some stage of reproduction. Here also the spores were found to have a capsule or cyst about them. The capsules are spherical and no larger than the long axis of the spores. The protoplasm first segregates in a thin deeply staining rim about the inner side of the capsule, although a single spherical mass is always left within. (Figure 2.) This mass I consider homologous with the protoplasm (or possibly nucleoplasm) left over after sporozoite formation in Coccidia or Gregarinida (Reliquat de différentiation). The peripheral protoplasm next fragments into eight spheres or sporozoites similar in all respects to those from the intestinal spores. The sporozoites break through the capsule and collect around the outside (Figure 1, A, B, C, D, E, H, I), while empty spore cysts are often seen with minute apertures (Figure 1, F, G).

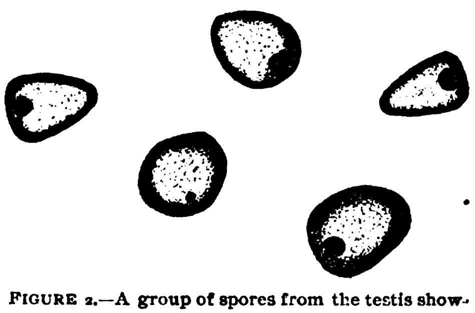

FIGURE 2.—A group of spores from the testis showing preparatory stages in sporozoite-formation. Cf. Figure 1. Camera drawing, x 4000 diameters.

The spores and sporozoites in the intestine and in the testis are apparently identical with the single exception of the cyst membrane or capsule. The absence of a membrane in the intestinal forms is undoubtedly due to its dissolution by the various digestive fluids of the alimentary tract, while in the testis there are no such solvents,

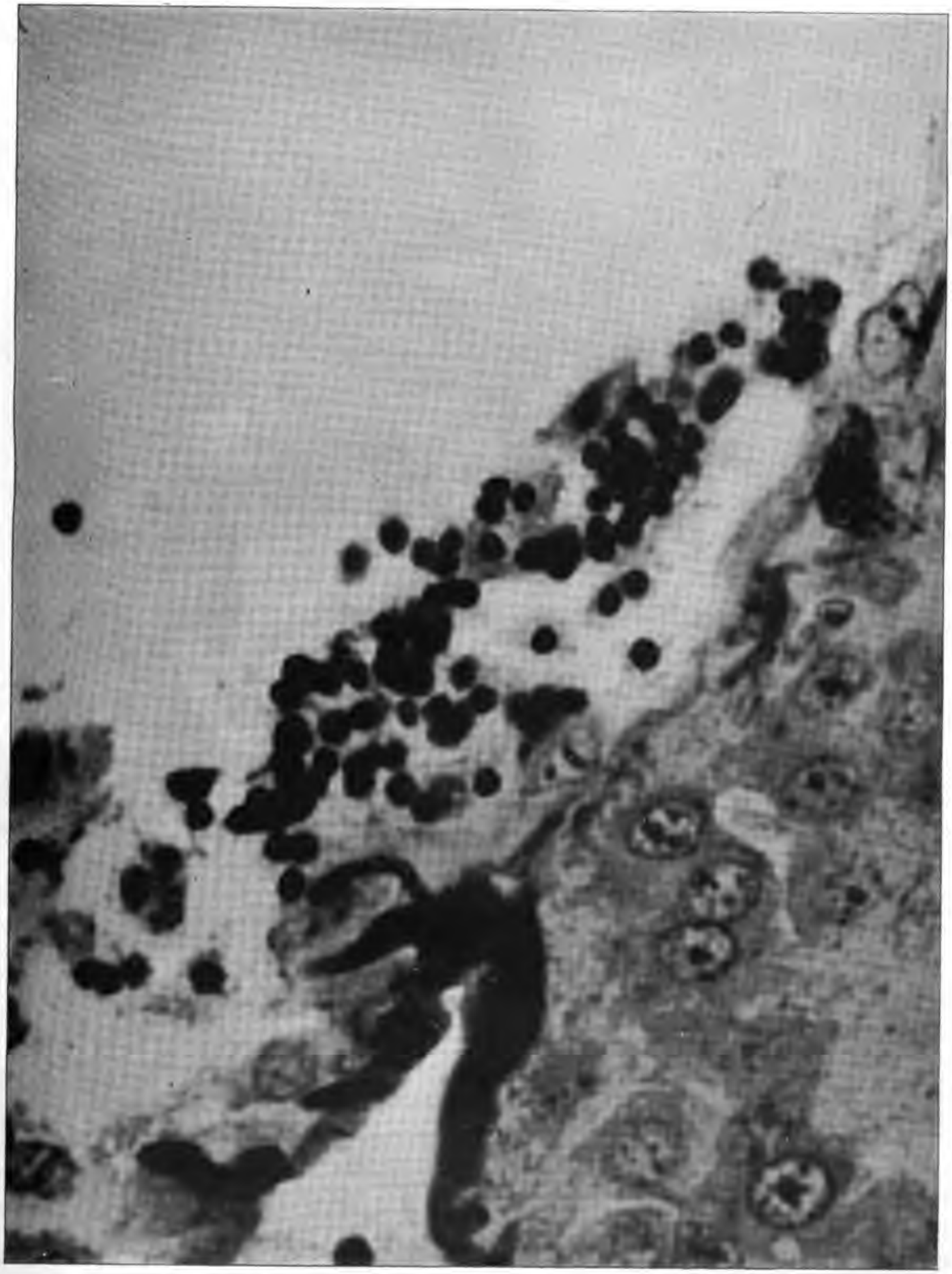

PLATE V.—SPORES IN THE LYMPH SURROUNDING THE LIVER.

the sporozoites escaping by rupture of the cyst. In no case were there thread-bearing capsules as in the spores of the Myxosporidia.

A nucleus could not be made out either in the spore or the sporozoite. The entire cell, however, acts like a nucleus in its staining reactions and I believe that neither the spore, the sporozoite, nor the adult organism has a morphological nucleus, but all possess chromatin distributed throughout the cell.

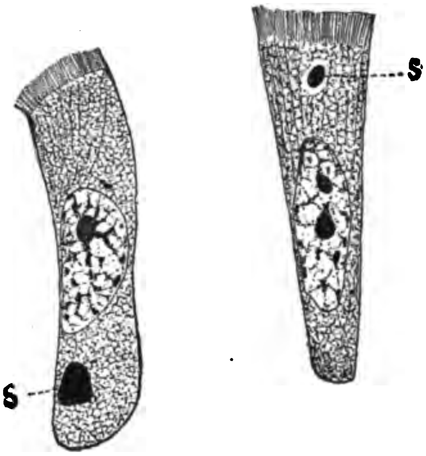

FIGURE 3.—Two epithelial cells from the pyloric cœca showing the sporozoites (S) in the cytoplasm. Camera drawing, x 2000 diameters.

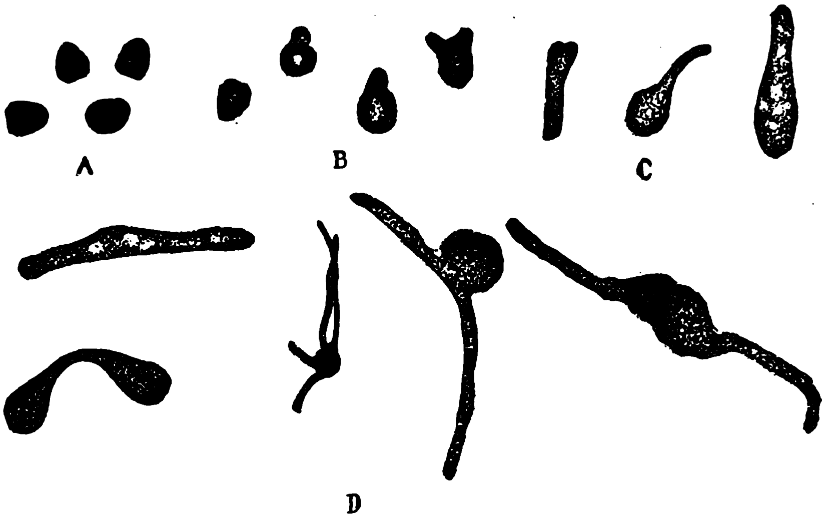

FIGURE 4.—Transformation of the sporozoite and growth into the adult form. *A*, a group of four sporozoites as they appear in the lymph. *B*, the beginnings of pseudopodia—formation and appearance of the vacuole. *C*, loss of the homogeneous appearance and beginning of the reticular condition. *D*, later stages showing disappearance of the reticulate structure and the increase of the densely staining cytoplasmic granules. The last two forms are twenty-two and twenty-five microns in length. Camera drawings, x 2000 diameters.

No trace of a motile organ could be found on the sporozoites, which, in the intestine, were lost in the hordes of bacteria, so that they could not be followed. In the testis, however, they were found in groups against the epithelial tissues and were often seen in the epithelial cells. In the digestive tract they also reach the epithelial cells and as intra-cellular parasites grow to homogeneous masses of about the same size as the spores (Figure 3). In this stage of growth it is impossible to tell, except by their further history, the sporozoite from the spore (Figure 4, A). As a rule, however, the sporozoites are common in the lymph around the intestine, pyloric cœca, etc., and they usually show some indication of amœboid motion which is never

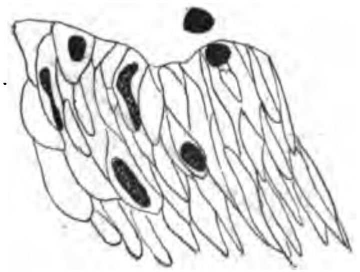

FIGURE 5.—Sporozoites entering muscle cells from the lymph space. Beginning of the amœboid stage. Camera drawing, x 1400 diameters.

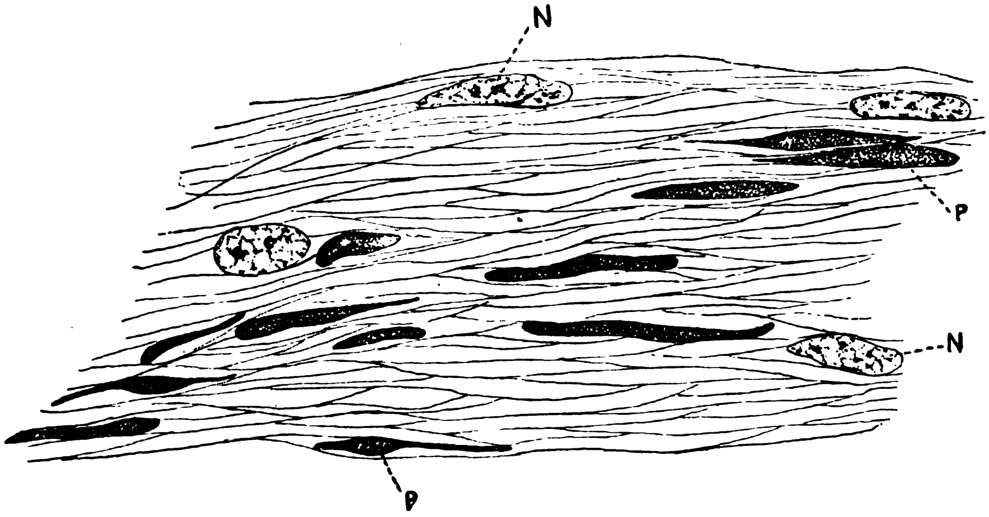

FIGURE 6.—Unstriped muscle fibres from the walls of a pyloric cœcum, containing the adult parasites (*P*) in the amœboid stage. *N*, *N*, nuclei of the muscle fibres. Camera drawing, x 1400 diameters.

observed in the spore. They get into the lymph through the epithelial cells and here develop rapidly into amœboid forms, especially in the vicinity of the unstriped muscles surrounding the digestive tract. The first indication of this change is a small vacuole in the center of the sporozoite (Figure 4, B). This is almost always accompanied by a minute swelling at one end of the organism (Figure 4, B), and the swelling becomes a pseudopodium (Figure 4, C). The amœboid condition is then established and in this state the young parasite penetrates a muscle bundle and takes a position among the fibres. As it changes from the sporozoite into the amœboid form, the dense homogeneous appearance is lost and it becomes first vacuolated and then finely reticular in structure. In still later stages the protoplasm becomes densely granular and the reticulum difficult to make out. The parasite in the amœboid stage frequently reaches a considerable size, although I am not able to say whether the larger forms have been in muscle cells and have emerged preparatory to spore formation or have developed in the lymph. I am inclined to the former alternative because of the densely granular protoplasm and because the only parasites that I have seen entering the muscle tissues were smaller forms. The largest amœboid individuals (Figures 4, D, and 7, B, D) measure from eighteen to twenty-five microns, while the sporozoites entering the muscle cells measure only from two to four microns (Figure 5). Here,

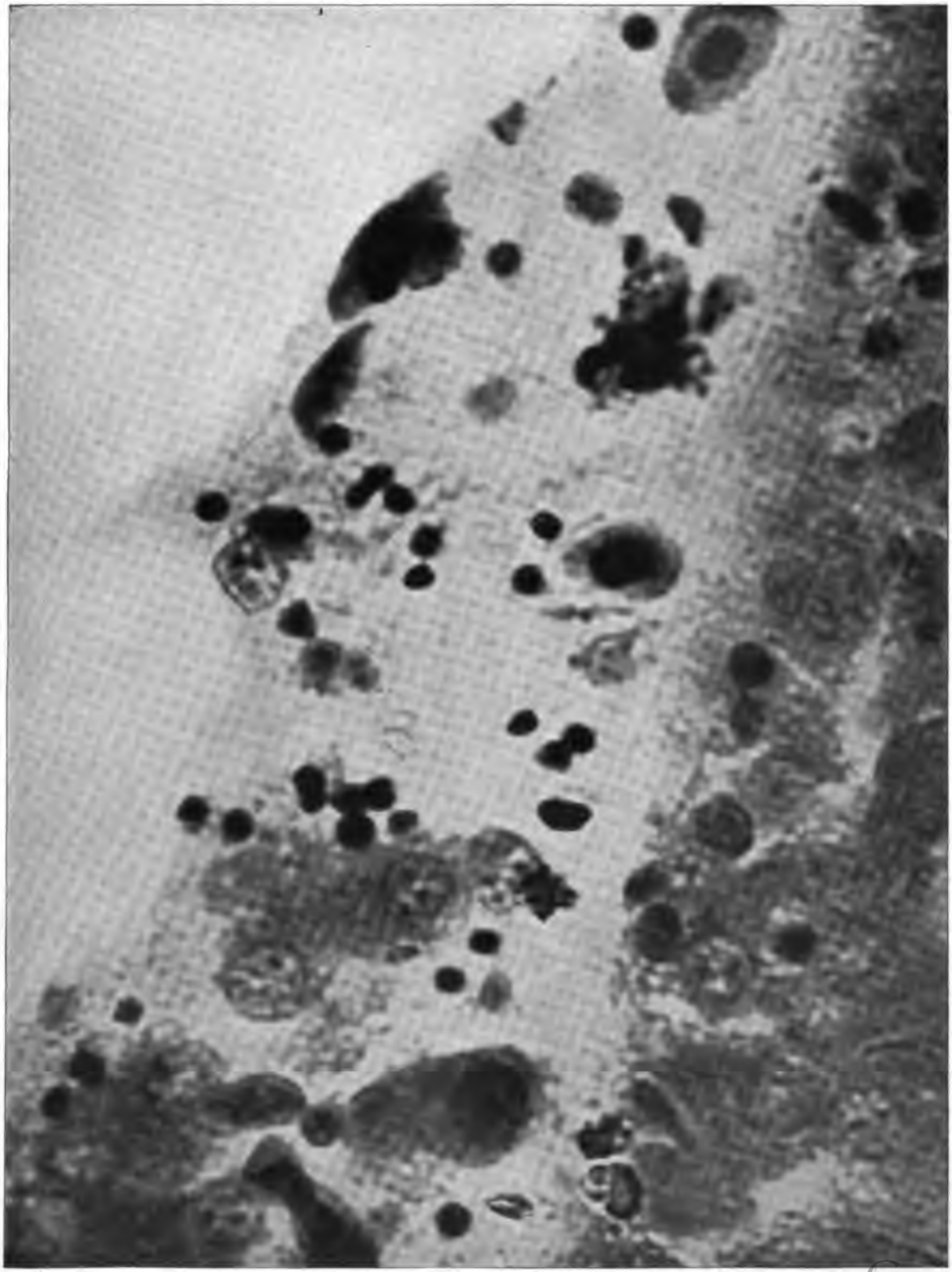

PLATE VI.—SPORES IN THE LYMPH SURROUNDING THE KIDNEY.

however, they rapidly grow in size until they attain their largest dimensions (twenty-five microns). While the amœboid forms assume any characteristic shape in the lymph, they have only one general form in the muscle cells. Here, constrained apparently by the tightly pressed muscle fibres, they are always elongate, sometimes fusiform, sometimes club-shaped (Figure 6). They can always be distinguished from the nuclei of the muscle cells by their characteristic shape and by their densely granular plasm.

Here, as in the spores and sporozoites, a nucleus could not be distinguished. But the deeply staining granules appeared like chromatin and in the older individuals they were grouped together in small aggregates which formed the beginning of the spores (Figure 7, D).

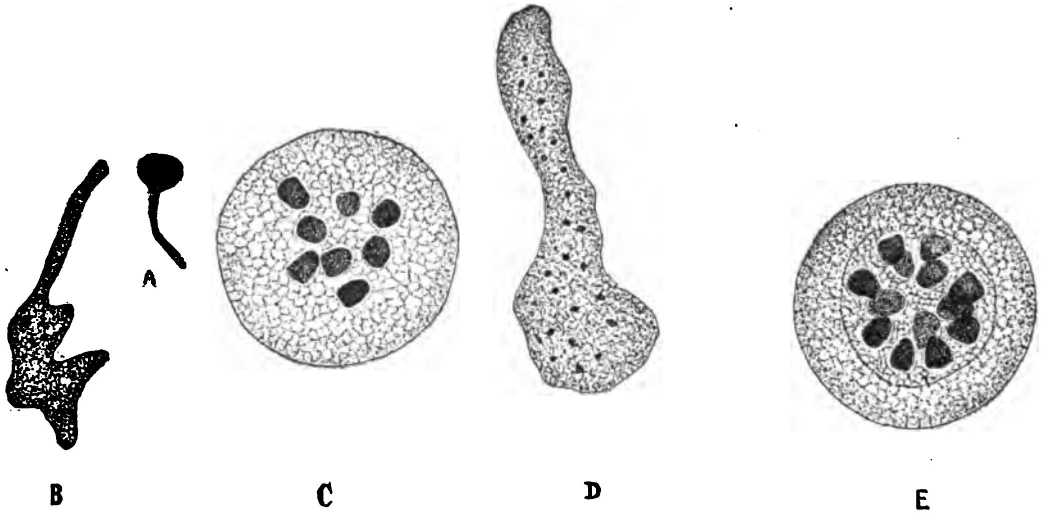

FIGURE 7.—Spore formation. *A*, Young sporozoite at the beginning of the amœboid stage. *B* and *D*, large amœboid forms prior to spore formation. In *D* the deeply staining granules have begun to collect in groups. *C* and *E*, spore-forming cysts. The protoplasm is again reticulate and the spores are completely formed. Camera drawing, x 2000 diameters.

Spore formation is always preceded by encystment of the animal within a delicate membrane. The cell leaves the muscle tissue, and in the lymph of the body cavity it rounds out into a sphere. The amœboid individuals, when ready to form spores, are comparatively large, and the cysts are of variable size, in some cases measuring twenty microns or more in diameter. The spores are formed by aggregation of the deeply staining granules (chromatin?) instead of by nuclear division as in other Sporozoa (Figure 7, B, D). This leaves the protoplasm with a clearly marked reticular structure as in the early stages (Figure 7, C, E). A variable number of spores is the rule. In some cysts only twelve were seen, in others sixteen or even more. In some cases the cysts appear to be differentiated into a more hyaline ectoplasmic, and a denser endoplasmic region, the spores being in the latter (Figure 7, E). A more or less definite membrane separates the two regions. This membrane is not obligatory, however, and is often wanting.

The spores thus formed are liberated into the body cavity and are carried to all parts of the body with the lymph and blood. Whether they are voided to the outside or not, I do not know. In some cases the cysts, which are most often found in the body cavity, were observed in the intestine and I can account for their presence here on the assumption that they had been taken in as food or else derived from the gall bladder, for in no case have I found amœboid stages in the digestive tract. If they are voided to the outside it is probably by way of the gall bladder, for in this organ they were very abundant.

Briefly summarizing the above observations it appears that the parasites are 1) taken into the digestive tract with the food either in the cyst (adult) stage or in the spore stage; 2) the spores form sporozoites in the stomach and intestine; 3) these penetrate the epithelial cells and work their way to the lymph spaces; 4) in the lymph they develop amœboid processes and then penetrate muscle bundles; 5) here they grow to the adult stage, becoming comparatively large amœboid organisms of spindle or club shape; 6) they return to the lymph and there round out into spore-forming cysts; 7) the spores are apparently formed by the segregation of chromatin (?) granules which leave the remaining protoplasm with a distinctly reticular structure; they are variable in number; 8) the spores are liberated in the body cavity from which they find their way to all parts of the body, but accumulate especially in the testis. Or the cysts may be voided to the outside by way of the gall bladder; 9) in the testis these spores form sporozoites and thus lead to auto-infection. These spores, unlike those in the intestine, are covered by a capsule.

I am not satisfied to conclude, however, that the entire life history of the parasite is to be found in the trout. The absence of all traces of conjugation leads me to believe that some important stages in the life history are passed in some hosts other than this fish. I may digress from my immediate subject long enough to point out a case which illustrates this point and which has only recently been made known through researches of Major Ross in India, of Professors Koch, Grassi and others in Germany and Italy. The malaria-causing organism in man (*Plasmodium malariæ*) is a parasite belonging to the same group (Sporozoa) as the fish parasite here considered. Its life history was not completely known until within the last two years, when it was discovered that a very important stage in its development is passed in the digestive tract of a mosquito. Professor Koch and other scientists in Germany found that the malaria germs which are taken with the blood into a mosquito's stomach, conjugate there in pairs, a male germ fusing with a female. The copula which is formed by

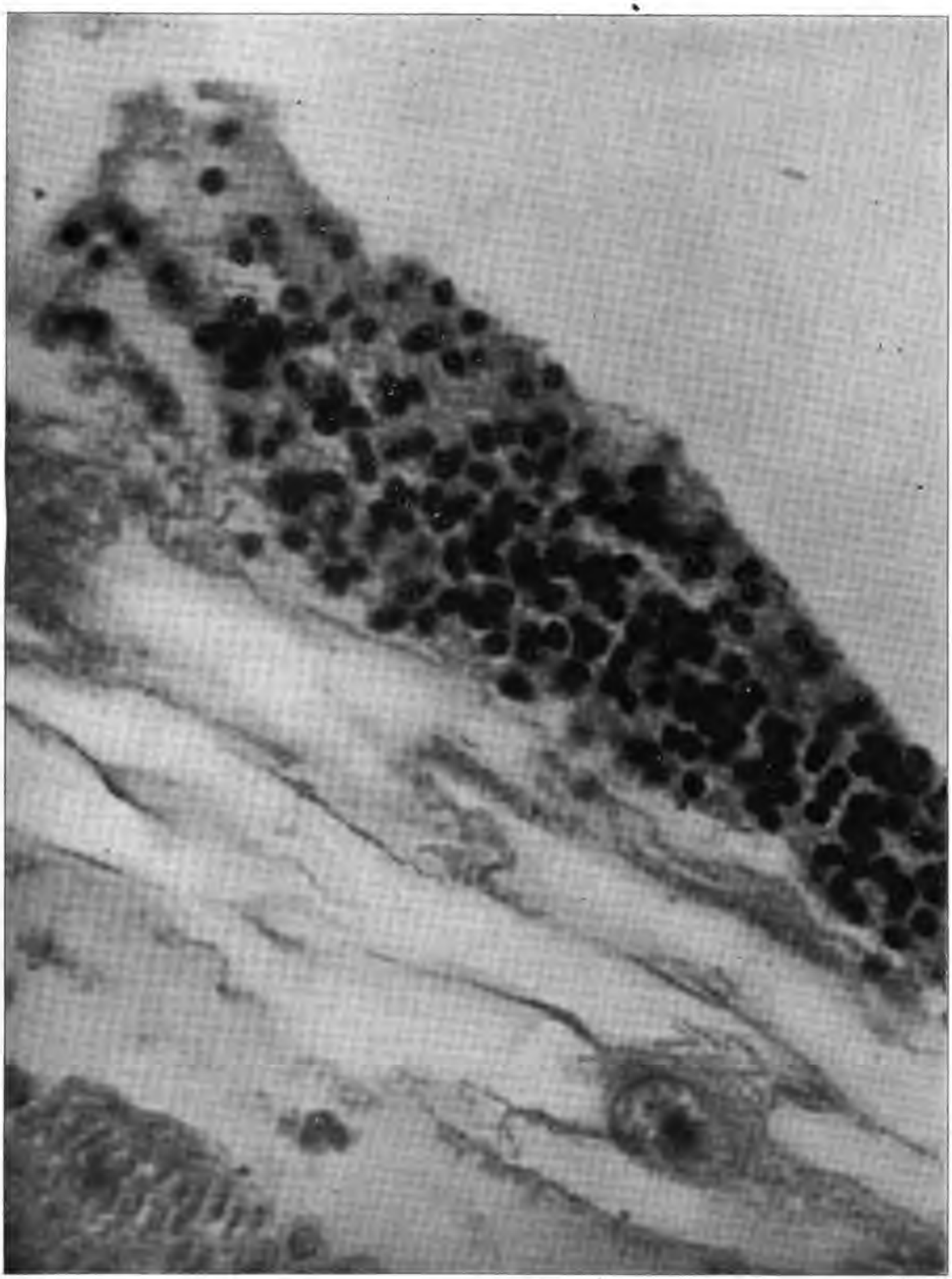

PLATE VII.—MASS OF SPORES ON THE OUTSIDE OF THE INTESTINE.

this union penetrates an epithelial cell of the gut where it forms spores. These spores migrate into the body cavity of the insect, ultimately finding their way into the salivary glands, and are injected into the blood of a new human host when the proboscis of the mosquito is forced into the flesh. Here is a change of hosts which are widely separated in the animal scale, one a warm-blooded vertebrate, the other an inferior invertebrate with body fluids of an entirely different nature.

A similar change of hosts may occur in other forms of Sporozoa as well as in the malaria germ, and although I do not want to insist upon it, it is certainly possible that the parasite which is causing the present epidemic is only one phase of some organism which is parasitic in some other form of animal life as well. What that form may be I have no means of knowing. The most probable hosts would be looked for among the arthropods such as flies, small crustacea, water beetles, and larvæ of various kinds or worms of various sorts. The arthropods are the most widely distributed hosts of Sporozoa while worms are almost equally affected.

On the other hand it may be possible that the form under consideration is a permanent parasite of the fish, becoming pathogenic only when the means of resistance of its host are weakened enough to permit it to increase to large numbers. Pfeiffer* regards this as a possible explanation of the epidemics among the barbels from the Rhine, Moselle and Saale, which are caused by the allied forms of Sporozoa, the Myxosporidia. I was unable to find them, however, in a presumably healthy fish from another part of the State and regard this view as improbable, although I am not prepared to say that they are not present in healthy fish in Long Island waters. The presence of the parasite in the intestine of the trout indicates that this is the means of infection from host to host, rather than through the gills where I found none, or directly from the outside through the skin. The vital question is: What is the original source of infection? This question, I regret to say, cannot be answered at the present time. The organism is newly discovered and its affinities are very uncertain. The nearest approach to it are the forms described by Pfeiffer† as Serosporidia. These are minute parasites occupying the body cavities of various Crustacea (*Daphnia*, *Gammarus*, *Cypris*, several species). Their form is spherical, oval or pyriform, and from four to ninety microns in diameter. The protoplasmic body is finely granular. Reproduction takes place in two ways: either the parasite changes into a cyst the contents of which break up into numerous amœboid spores, or it divides. The method of infection and the general distribution of these forms are quite unknown. The full life history of these Crustacean parasites is also unknown. It may be pointed out, however, that the hosts in which these parasites live are minute fresh-water forms which may easily be swallowed by the fish. The present parasite also resembles the form described by Thélohan and Henneguy* as a parasite of the crayfish, and although the spores of the latter do not contain thread capsules the authors regard it as one of the Myxosporidia. On similar grounds the present parasite might be regarded as one of the Myxosporidia. After one fish is affected the epidemic may spread throughout the entire hatchery by secondary infection; in the case of Myxosporidiosis, Megnin (see Pfeiffer), Pfeiffer, Ludwig, etc., believe that secondary infection is brought about by the extensive fouling of the water by fish corpses, and they recommend careful prophylactic measures such as removal of dead fish and weeding out of infected fish showing external signs of the disease. The most careful precautionary measures, however, cannot prevent a certain amount of contamination of the water from ulcers of infected fish.

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* Protozoen als Krankheitserreger. Edition 1891.

† Sporozoen als Krankheitserreger. Edition 1895.

Summary.

The cause of the disease can be safely assumed to be a minute parasite, *Lymphosporidium truttæ,* belonging to a great group of unicellular parasites, the Sporozoa. Its closest allies are the Serosporidia which infest and block up the body cavities of certain fresh-water Crustacea (*Cypris,* etc.). It forms sporozoites in the digestive tract of the trout; these penetrate epithelial cells and grow to forms similar to those in the intestine. They finally make their way into the lymph spaces and body cavity (probably by amœboid motion), where they penetrate muscle cells. No cysts comparable with those of the Myxosporidia are formed, but, as in the Myxosporidia, there is an amœboid adult stage which forms spores. Primary infection probably takes place by ingestion of the parasite, but whether these are in the free state or are parasites in some other hosts which are eaten as food, could not be determined. Secondary infection undoubtedly takes place by contamination of the water by dead fish and from ulcers on infected fish.

The cause of the epidemic at this particular time cannot be ascertained. I have every assurance from the director that the water was pure in every respect and never rose above sixty degrees Fahrenheit in temperature, while a constant flow from springs kept it fresh. Furthermore, his statements that interbreeding was not allowed and that fresh material was introduced through eggs and milt from other parts of the State leaves no reason for regarding the epidemic as due to the lack of vitality through this cause.

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* Ann. d. Micrographie, 1890.

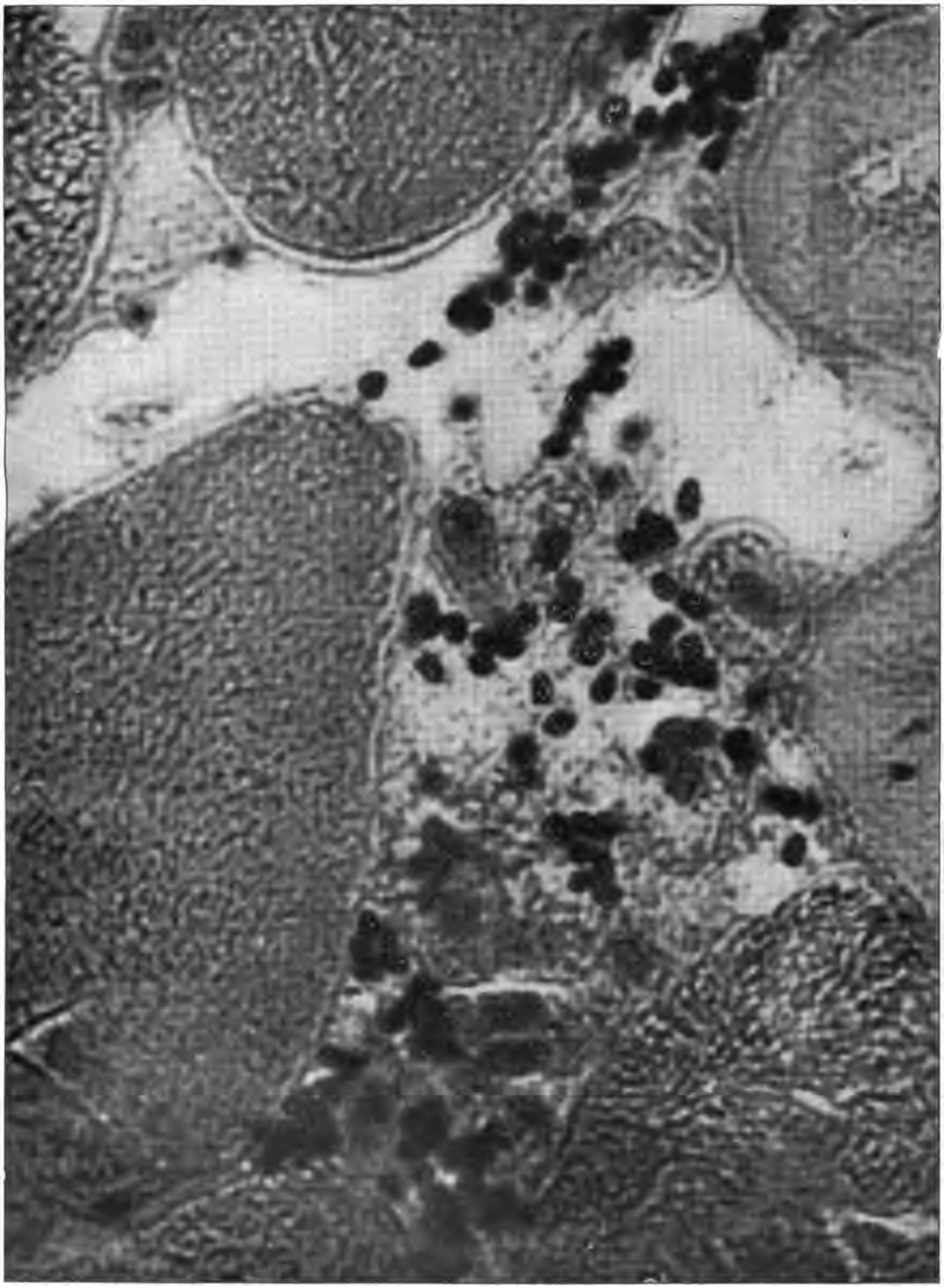

PLATE VIII.—SECTION OF MUSCLE BUNDLES OF THE BODY WALL.—SPORES IN THE LYMPH SPACES.

Recommendations.

My attention was first called to the epidemic in October, 1899, after it had been running for a period of five months and after thousands of fish had died. It was quite evident that the disease was then widespread and that nothing could be done to save the remainder. The question to be considered is how to prevent future outbreaks of like nature in this and in other localities. Unfortunately this cannot be answered until we know where the parasite lives when not in the body of the fish. A systematic examination of the suspended organisms in the waters, such as *Cypris, Daphnia, Gammarus, Cyclops* or other Crustacea, might throw some light on the question, but until this is ascertained the only recommendations that can be made are such as the director of the Northport hatchery has undoubtedly carried out in the present instance, viz.: 1) to exercise the most careful prophylactic measures. As soon as any fish shows the first evidence of the disease it should, of course, be removed and buried or burned (Pfeiffer, Ludwig and others recommended these methods in the case of Myxosporidiosis), the remaining fish should all be inspected at intervals and all sickly ones should be removed. 2) In the present case, before stocking the ponds anew, I should recommend that the water be drained off and the bottoms be left exposed to the sun for a few months. The runways should be scrubbed and all growths removed. Every fish now in the ponds should be removed and not allowed to contaminate the fish in neighboring streams. 3) Care must be taken not to interbreed with the diseased fish, for although it is perhaps improbable that the disease germ is transmitted in this way, yet it is possible, especially as the testis is the chief seat of reproduction of the parasite. 4) The water must be kept perfectly clean and cold; not only dead fish but refuse of all kinds should be carefully removed. 5) The vitality of the fish must be sustained; fungoid growths must be constantly watched for and removed. 6) Constant interbreeding of the same limited variety of fish should be avoided; new blood should be introduced frequently. 7) The food of the fish should be carefully inspected and should not be allowed to stand exposed to flies and other insects, but should be fresh.

These homely recommendations, which every fish breeder knows and probably applies, are the only precautionary measures that I can suggest, and even where they are most rigorously enforced the disease may spread until it becomes epidemic. The fish breeder must add this newly discovered organism to the many chances he takes in rearing fine fish.

DEPARTMENT OF ZOOLOGY,
COLUMBIA UNIVERSITY, NEW YORK.

Description of the Photographic Plates.

The photographs represented in the following plates were made by Dr. Edward L. Leaming, of the Department of Pathology, Columbia University, New York:

Plate I. A diseased trout, showing loss of the lower jaw bones. The lower figure represents a gill from an adult fish covered with the parasitic Copepod *Tracheliastes*. Photograph magnified one and one-half diameters.

Plate II. Two diseased trout, showing ulcers in the body wall. The testis is seen protruding through the anterior hole in the lower fish. Magnified one and one-quarter diameters.

Plate III. A group of spores of the parasite *Lymphosporidium truttæ* from the intestine. Some rod-bacilli are seen near the center. Microphotograph, x 2000 diameters.

Plate IV. Section of the testis to show the degree of infection. The black granular parts are groups of spores of the parasite, the light parts are the epithelial tissues of the testis from which the stain has been entirely extracted. Attention is directed also to the layer of spores around the left margin of the section. Microphotograph, x 80 diameters.

Plate V. A group of spores in the lymph surrounding the liver. Microphotograph, x 2000 diameters.

Plate VI. A group of spores in the lymph surrounding the kidney. Microphotograph, x 2000 diameters.

Plate VII. A group of spores from the body cavity and in the lymph surrounding the intestine. Microphotograph, x 2000 diameters.

Plate VIII. Spores in the lymph spaces between the muscles of the body wall. Microphotograph of a section from an ulcerated spot, x 2000 diameters.

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